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The neuromatrix and the epileptic brain: behavioral and learning preservation in limbic epileptic rats treated with ketamine but not acepromazine

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Abstract

Conceiving the organization of the brain as a "neuromatrix" could provide significant insights into how different injuries to the nervous system could result in very distinct changes in behavior. The use of different pharmacological treatments to combat the deleterious consequences of such injuries is common practice. However, such treatments may have the capacity to alter the configurations of various neuronal circuits that contribute to the "neuromatrix" by selectively preventing damage to some pathways while facilitating the spread of destruction along others. The choice of pharmacological treatment may have profound consequences on the recovery of normal functioning following injury. We examined the behavior of rats treated with one of two potentially neuroprotective agents, the *N*-methyl-D-aspartate antagonist ketamine and the atypical neuroleptic acepromazine, on seizures induced by lithium–pilocarpine. Rats treated with ketamine following seizure onset were virtually indistinguishable from nonepileptic controls on a variety of behavioral tasks that included tests on learning, memory, and anxiety. In contrast, acepromazine-treated rats showed marked deficits on all learning and behavioral measures tested. These results suggest that administration of ketamine relatively soon after the emergence of epilepsy can prevent many of the cognitive deficits that are commonly found in rats subjected to lithium–pilocarpine-induced seizures. Further clinical testing investigating ketamine as a potential adjunct treatment for epilepsy may be well warranted.
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1. Introduction

A basic assumption of the *neuromatrix hypothesis* is that the specific choice of pharmacological agent, when administered shortly after the onset of a brain injury, can significantly influence the severity and pattern of multifocal neuronal damage. Functionally distinct mosaics in patterns of damage could potentially serve as additional or alternative substrates for neuronal processing through which the conspicuous appearance of "normal" behavior can still be maintained despite evidence of extensive brain damage. It is therefore important to consider that pharmacological manipulation at very specific points in time (occurring relatively soon after the initial injury) may have the potential to influence the expression of these developing mosaics of damage in such a manner that the preservation of behavior can be either maintained or significantly altered.

Temporal lobe epilepsy is a chronic disorder that can profoundly influence numerous aspects of both cognitive and behavioral functioning. The neuropathological and behavioral consequences commonly associated with this form of epilepsy can be effectively reproduced in rodents [1]. The lithium–pilocarpine model has served as an experimental model for the induction of temporal lobe epilepsy and produces many of the histopathological and cognitive changes that are found in human

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forms of temporal lobe epilepsy [2–4]. The mortality rate associated with pilocarpine-induced seizures is considerably high (greater than 95% mortality during the first 2 days following status epilepticus); however, with appropriate pharmacological treatment animals can survive long enough to develop many of the pathological features that are found in human epilepsy, including extensive cell loss or dropout, mossy fiber synaptic reorganization, spontaneous recurrent seizures, and pervasive cognitive and behavioral deficits following status epilepticus [4,5].

Previous studies in our laboratory [6–10] have shown that administration of either the neuroleptic acepromazine or the noncompetitive *N*-methyl-D-aspartate (NMDA) antagonist ketamine immediately following the emergence of status epilepticus can produce reliable differences in the gross pattern of neuronal dropout and necrosis despite a similar extent (or percentage) of multifocal neuronal injury. These studies demonstrate that although a similar amount of brain damage is observed in each of these conditions, a profound alteration in both behavioral and learning processes follows that is highly dependent on the unique choice of the pharmacological agent given following the onset of the seizure.

Rats treated with acepromazine (a D1 antagonist) following seizures have marked behavioral impairments (enhanced aggressiveness toward conspecifics and handlers, self-mutilation, hypersexuality), attenuated learning, and disrupted memory processes (i.e., radial maze learning, conditioned taste aversion), as well as the proclivity toward bouts of "spontaneous" seizures [8,11–14]. On the other hand, ketamine-treated rats have demonstrated no observable behavioral abnormalities or learning impairment and essentially resemble nonseized control rats in a variety of tested paradigms despite the presence of extensive multifocal brain injury [8–10]. These results suggest that a behavioral continuum may exist in which ketamine- and acepromazine-treated animals occupy opposite extremes in terms of behavioral and learning functioning despite a similar extent of brain damage. This further implies that pharmacological manipulation early after the onset of the seizure may have the potential to selectively preserve some neuronal circuits while selectively damaging others. This "neuronal darwinism" implies that only the strongest and most adaptable connections that are recruited during the initial seizure episode will remain. Alternatively, the weaker and more susceptible neuronal circuits that are most affected by the accompanied excitotoxic processes during seizure induction will be more readily damaged or severely impaired. The outcome of this may be differences between the potential combinations of inhibitory and excitatory connections that mediate neuronal representations of normal behavior.

The lithium–pilocarpine seizure model, with its capacity for generating extensive partial neuronal loss within various neuronal pathways, can provide the necessary conditions to serve as a model for investigating the features of the neuromatrix hypothesis. It can also determine how specific receptor-based pharmacological manipulation can significantly perturb the expression of advancing injury and the extent of deficits to different functional neuronal circuits that mediate behavior (such as those circuits that are important in learning and anxiety). The emergence of different mosaics or patterns of brain damage that are facilitated by different drug interactions with various receptor systems could lead to the production of a variety of qualitatively distinct types of emergent behaviors, some of which may even be considered "pathological." Even more interesting is that the individual could even respond to or perhaps even experience the same stimulus in a different manner following the reconfiguration of neuronal connections that was influenced both by the initial injury and by the choice of pharmacological treatment. Moreover, these mosaics of brain damage may even provide additional or alternative routes for *preserved* and *normal* behavioral functioning despite extensive and pervasive multifocal neuronal loss.

To address some of the basic features of the neuromatrix hypothesis the following study was designed to evaluate the extent of learning and behavioral preservation (or its conspicuous change) following pilocarpine-induced seizures in rats that were treated with either acepromazine or ketamine. Rats were tested in the open-field, elevated-plus maze, and contextual fear conditioning paradigms. Furthermore, an additional group of epileptic rats, treated with either ketamine or acepromazine were tested for defensive reactivity to capture in a paradigm adapted from Albert and Richmond [15].

2. Methods

2.1. Subjects

A total of 27 male albino Wistar rats, approximately 70–90 days of age and weighing between 300 and 680 g, were used in the following experiment. All animals were housed three or four per cage in either standard metal cages or plastic shoebox cages during the entire duration of the study. Food and water were available ad libitum. The light:dark cycle was 12:12 with photophase onset at 0730 hours local time and with the ambient temperature maintained between 20 and 21 °C. Behavioral testing took place during the early to midphotophase period. All procedures were approved by the local Animal Care Committee and were in compliance with the Canadian Council for Animal Care (CCAC) established guidelines.

2.2. Seizure induction

After a week of habituation to colony conditions, rats received either a subcutaneous injection of lithium chloride (3 mEq/kg) followed 4 hours later by a single systemic injection of either pilocarpine (30 mg/kg) or saline. This combination of lithium pretreatment has previously been shown to reduce the dose of pilocarpine required to evoke convulsions by a factor of about 10. On receiving the injection of pilocarpine, rats were closely monitored for the onset of stage 4/5 motor seizures [see 16] and the time or latency (in seconds) to seizure onset was recorded for each animal. Within 5 minutes of the first overt behavioral sign of seizure induction (i.e., status epilepticus), rats received either a 100 mg/kg subcutaneous injection of the noncompetitive NMDA antagonist ketamine (Sigma, St. Louis, MO, USA) or a 25 mg/kg subcutaneous injection of the dopaminergic antagonist acepromazine (Ayerst Laboratories, Montreal, Canada). All drugs were given at a volume of 1 cc/kg.

We did not include a non-status epilepticus control group (i.e., a group that received acepromazine or ketamine but not lithium–pilocarpine), since there is no evidence that acute treatment with ketamine or acepromazine at the doses employed in this study can produce any acute neurotoxic effects or discernable necrosis under light microscopy [7,17,18]. (Our dose for both of these drugs was within the range that is commonly used in veterinarian practice for surgical protocols.) Because of ethical considerations a pilocarpine/saline group was also not included in this study as more than 95% of these animals invariably die within the first 24 hours following seizure induction [7].

After seizures were induced, each rat was returned to the respective home cage, closely monitored, and given standard methods (rat chow mixed in a water solution) to facilitate recovery [7]. Since acepromazine-treated rats can often show extensive aggression to conspecifics when caged in grouped conditions, rats were inspected for physical damage (i.e., lesions) nightly at the same time during the course of the study. After a 2-week recovery period, rats that survived the seizure induction process were tested using several behavioral paradigms which included open-field, elevated-plus maze, resistance to capture, and contextual fear conditioning testing.

2.3. Open-field testing

The open-field test was used as an evaluation of behavioral and motor responses of rats to a novel environment [19]. The open-field testing box was a custom-constructed square box (60 cm × 60 cm × 30 cm deep) that was subdivided into 25 approximately equal squares by black lines. Immediately on placing the subject into the center region of the open field, movements and accompanied behaviors were scored for a 2-minute observational period. Spontaneous locomotive behavior was defined as the number of squares crossed by both hind legs during the observational period. In addition, the numbers of rearings on hind feet and grooming episodes were recorded. The rats were also quantified on defecation and micturition during the 2-minute observational period. Between subjects, the open field was cleaned using a diluted 10% ethanol cleaning solution.

2.4. Elevated-plus maze

The elevated-plus maze has been considered a measure for evaluating an ethologically derived animal model of anxiety behavior [20]. The elevated-plus maze consisted of two open arms, 52 × 12 cm, and two enclosed arms, 52 × 12 cm, surrounded by 40-cm-high walls with an open roof, arranged in such a manner that the two arms of each type were opposite each other. The maze was elevated to a height of 80 cm above the floor.

Subjects were individually placed in the center of the maze (the platform center) directly facing one of the open arms. A number of classic parameters were measured during a 5-minute observational period, including: (1) open arm duration—the total amount of time spent in the open arms; (2) closed arm duration—the total amount of time spent in the closed arms; (3) center platform duration—the total amount of time spent in the central platform; (4) open arm entry—the frequency of rat entry with all four paws into the open, unprotected arms; and (5) closed arm entry—the frequency of rat entry with all four paws into the closed, protected arms.

The ratio of open to total arm entries, a measure of relative aversiveness of the open arms and elevated places, was analyzed. The total arm entries, an index of locomotor activity in pharmacological validation studies using rats and mice observed during a 5-minute testing period, were also recorded. In addition to these classic measures, the number of times the animal fell during the course of the 5-minute testing, as well as the frequency of defecation and urination, was recorded. The rats that fell off the plus maze were immediately placed back at their original position and additional units of time were allotted depending on the time it took for the fallen rat to be retrieved. Therefore all subjects, regardless of whether they fell or not, received a maximum of 5 minutes on the apparatus. On completion the subject was removed and quickly returned to its home cage. The maze was cleaned after each subject with a 5% diluted ethanol solution to control for possible olfactory cues.

2.5. Contextual fear conditioning

The fear conditioning paradigm and apparatus have been described in great detail elsewhere [47]. Briefly, rats

were placed in the conditioning chamber and allowed 3 minutes of baseline measurements within the context. Following this, three unsignaled footshocks were delivered through a metal grid floor (2 s, 0.5 mA, 60-s interstimulus interval). Sixty seconds following the last footshock, the subject was returned to the home cage. The intensity of the electric current administered was controlled with an A-615-C Master Shocker (Lafayette Instruments, Lafayette, IN, USA). During the baseline period (preshock period) spontaneous locomotor activity (defined as a forward movement across the chamber midline) was recorded. Following the 3-minute of baseline, an unsignaled mild footshock was applied and rats were scored for defensive freezing, a behavioral index of fear. Freezing was defined as a halting or termination of all movement except for movement of the musculature required for respiration [21].

Twenty-four hours later, an 8-minute extinction test employing a time-sampling procedure that measures immobility (*freezing* (1) or *movement* (0)) was performed. Measurements were time-sampled and taken every 8 seconds during the entire 8-minute extinction test for a total of 60 observations. These nominal freezing measures were converted to a percentage of total observations. After each subject was tested the conditioning chamber was cleaned using a 0.4% acetic acid solution to eliminate any possible olfactory confounds.

2.6. Resistance to capture

Twelve rats (postseizure treatments: acepromazine, $n = 4$; ketamine, $n = 4$; nonseized controls, $n = 4$) were allowed 5 minutes to habituate in the open-field apparatus (dimensions described in detail above). After the 5-minute habituation period, the rat was picked up by an experimenter who was wearing a leather glove. The rat's resistance to being picked up was scored on the following 7-point scale adapted from Albert and Richmond [15]: 0 = easy to pick up, 1 = vocalizes or shies away from experimenter's hand, 2 = vocalizes and shies away from experimenter's hand, 3 = runs away from the hand, 4 = runs away and vocalizes, 5 = bites or attempts to bite, 6 = launches a jump attack.

2.7. Statistical analyses

All analyses were completed using SPSS software loaded on a VAX 4000 computer. Between-group differences in behavioral measures were examined using one-way analyses of variance (ANOVAs) with post hoc analysis (Scheffe's tests). Multivariate analyses of variance (MANOVAs), with appropriate pair-wise *t* tests as post hoc tests, were employed to determine the direction of group differences on behavioral measures. The resistance to capture measure was assessed using nonparametric Kruskal-Wallis tests, followed by post hoc multiple comparisons. Group differences were considered statistically significant at the $p < 0.05$ level.

3. Results

Within 5 minutes of pilocarpine injection, all rats developed signs indicative and characteristic of cholinergic stimulation. These profiles often include: diarrhea, piloerection, excessive exploration, head nodding, chromodacryorrhea, salivation, and facial automatisms. Eventually this further progressed into bouts of overt limbic motor seizures that were characterized by a conspicuous rapid forepaw clonus, rearing, and falling motor sequel that are analogous to type 3–5 kindled seizures on the Racine seizure classification scale [16]. The latency to this motor seizure manifestation was approximately 1041 ± 181 seconds (approximately 17 minutes) and this time point was defined as the beginning of status epilepticus. Following lithium–pilocarpine pairing and accompanying status epilepticus, two rats that were treated with acepromazine died (2 of 10) within 48 hours despite preventive measures. Furthermore, none of the rats that were treated with ketamine ($n = 8$) died during the first 48 hours following seizure induction. Nine additional rats that received an injection of lithium chloride (3 mEq/kg, sc) followed 4 hours later by an injection of physiological saline served as a nonseized control group. None of these control rats displayed the typical behavioral sequel observed in pilocarpine or electrically kindled seizures. Interestingly, all of the rats that received acepromazine as a postseizure treatment began to develop spontaneous motor seizures around Days 10–15 after initial seizure induction. Ketamine-treated rats never displayed any behavioral indication of recurrent seizures during nightly observations or handling.

3.1. Contextual fear conditioning

A one-way analysis of variance (ANOVA) discerned no statistically significant differences in midline chamber crossovers between any of the treatment groups [$F(2, 22) = 0.73$, n.s.]. Furthermore, no significant differences in the average amount of postshock freezing were found among the various treatment conditions on the conditioning (training) day [$F(2, 22) = 0.31$, n.s.], suggesting that all rats responded in a similar fashion to the administered shock. The duration of freezing observed during the 8-minute extinction test revealed that seized rats treated with acepromazine froze significantly less compared with rats seized and treated with ketamine or those that served as nonseized controls [$F(2, 22) = 14.94$, $P < 0.001$, $\eta^2 = 0.58$]. No differences in freezing behaviors were observed between ketamine-treated and control animals. For comparison, the mean

percentages (SD) for the freezing observed during the 8-minute extinction test were 75.6% (±18.2) for ketamine and 42.9% (±16.8) for acepromazine-treated rats, and 81.8% (±11.0) for nonseized control rats. The correlation between latency to seizure onset was found not to be significant for the amount of freezing observed during the 8-minute extinction test ($r = -0.15$).

3.2. Open field

The number of square crossings was found not to differ significantly between groups [$F(2, 10) = 2.21$, n.s.] indicating that spontaneous baseline locomotor activity did not differ between any of the postseizure treatments. A MANOVA with one level repeated (observation segment) and one level not repeated (postseizure treatment groups) was performed to discern if there were any changes in spontaneous locomotor behavior during the entire 2-minute period of testing. When the observational periods were partitioned into 30-second intervals (resulting in four observational segments: 0–30, 30–60, 60–90, 90–120 seconds) no significant differences in locomotor behavior during any of the partitioned periods was observed [$F(6, 30) = 0.21$, n.s.]. All other behavioral measures recorded during the 2-minute observational period did not show any statistically significant effects [all Fs$(6, 30) < 1.60$].

3.3. Elevated-plus maze

The total amount of time spent in the open arms of the elevated-plus maze was shown to differ significantly between groups [$F(2, 22) = 7.87$, $P < 0.01$; $\eta^2 = 0.41$]. Post hoc analysis revealed that a significantly greater amount of time was spent in the open arms by acepromazine-treated rats compared with ketamine-treated rats and nonseized controls (Fig. 1A). The time spent in the closed arms was also found to differ significantly [$F(2, 22) = 9.26$, $P < 0.01$; $\eta^2 = 0.45$] between groups. According to post hoc analysis, acepromazine-treated rats spent less time in the closed arms compared with ketamine-treated rats and nonseized controls (Fig. 1A), which did not differ significantly from each other. Time spent in the central platform did not differ between the groups [$F(2, 22) = 1.38$, n.s.]. When the seizure onset time was correlated with the amount of time each rat spent in the open arms of the plus maze, a moderate negative correlation ($r = -0.42$; $\rho = -0.38$) was found that accounted for about 17% of the explained variance.

Greater numbers of entries into the open arms were observed in animals that were treated with acepromazine compared with all other groups [$F(2, 22) = 7.59$, $P < 0.01$; $\eta^2 = 0.41$]. Since the assumption of homogeneity of variance was violated [Bartlett's Box test for homogeneity of variance: $F = 4.16$, $P = 0.01$] and was only modestly improved by square root transformation

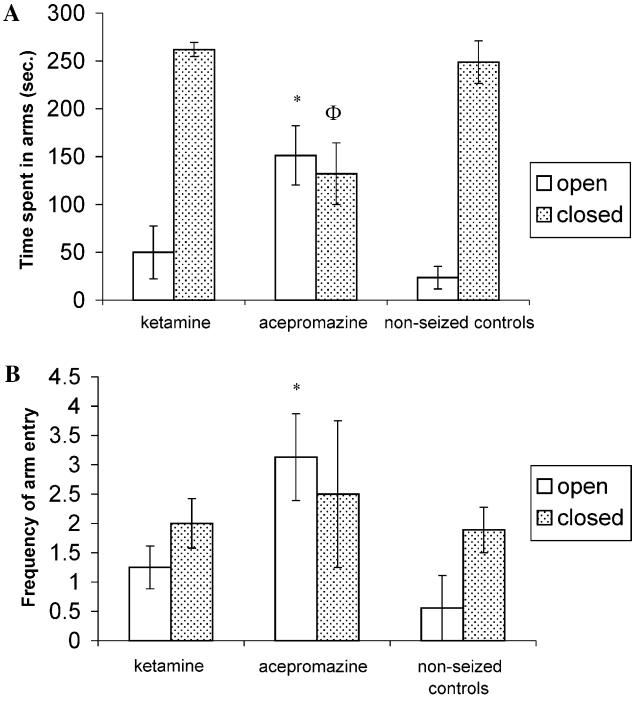

Fig. 1. (A) Time spent in the open and closed arms of the elevated-plus maze for ketamine-treated and acepromazine-treated limbic epileptic rats, and nonseized control rats. (B) Frequency of open arm and closed arm entries in the elevated-plus maze for ketamine-treated and acepromazine-treated limbic epileptic rats, and nonseized control rats. Error bars denote SEM. *Significantly different from ketamine and nonseized control ($P < 0.01$). ΦSignificantly different from Ketamine and nonseized control ($P < 0.05$).

of the data, a nonparametric (Kruskal–Wallis) test was performed on the numbers of open arm entries. The results confirmed the statistical finding that acepromazine rats made more open arm entries compared with all other groups [$\chi^2 = 9.16$, $P < 0.05$]. The number of closed arms entries was found not to be significant [$F(2, 22) = 0.17$; n.s.].

Fig. 1B illustrates the mean numbers of open arm and closed arm entries made during the 5-minute observational period on the elevated-plus apparatus. The relative aversiveness to the open arms was assessed by taking the ratio of open arm entries to total arm entries. This measure was found to be significant statistically [$F(2, 22) = 4.69$, $P < 0.05$; $\eta^2 = 0.31$]. Post hoc tests revealed that rats treated with acepromazine showed less aversiveness to the open arms compared with ketamine-treated rats or nonseized controls, which did not differ from each other. The total numbers of both open and closed arm entries, an index of nonspecific baseline motor activity, displayed only marginally significant differences between groups [$F(2, 22) = 2.95$, $P < 0.07$]. Interestingly, the numbers of times the rats fell off the plus maze during the 5-minute testing exhibited group differences [$F(2, 22) = 6.05$, $P < 0.01$; $\eta^2 = 0.35$]. Post hoc tests showed that animals treated with acepromazine

following seizure onset fell off (or jumped off) the apparatus more times compared with all other groups.

3.4. Resistance to capture

A possibility for the above finding that rats treated with acepromazine following seizure induction tended to fall off the elevated-plus maze apparatus significantly more times than all other groups could have reflected an increased motivation to escape the apparatus rather than a nonspecific locomotor deficit. To test this parallel or alternative hypothesis the resistance to being captured in a novel open field box was measured. The typical means (SEM) for the resistance to capture were 1.5 (±0.6) in nonseized control rats and, for the post-seizure groups, 3.2 (±0.5) in ketamine-treated rats and 4.8 (±0.5) in acepromazine-treated rats. A nonparametric (Kruskal–Wallis) test revealed a significant effect for resistance to capture score [$\chi^2 = 6.75$, $P = 0.034$]. Resistance to capture was found to be significantly higher in rats treated with acepromazine than in nonseized control rats. Ketamine-treated rats did not differ from acepromazine-treated rats with respect to resistance to capture. Although ketamine-treated rats did not differ from nonseized controls their resistance to capture score approached significance ($P = 0.08$).

4. Discussion

As stated earlier, the *neuromatrix* hypothesis [7,10] implies that during pivotal periods of intense neuronal activation (i.e., during a seizure) specific modulation of certain receptor systems can potentially influence the expression and pattern of brain damage that can occur. The end result may be specific deficits in both learning and memory functioning or the conspicuous maintenance of normal behavior. The emergence of a qualitatively observed novel behavior following such a pharmacological "reconfiguration" of the brain is one of the more compelling components derived from the hypothesis.

The "neuromatrix" hypothesis has been employed by Ronald Melzack to provide a conceptual understanding regarding the experience of pain [22]. Melzack proposed that the neural correlates of pain experience would be fundamentally influenced by both the "synaptic architecture" and the spatial distribution of neuronal aggregates within the brain [22]. These complex interactions between vastly interconnected neuronal populations would make up the foundation for the emergence of a neuromatrix. In other words, the interconnections between various neuronal elements within specific circuits can allow for the emergence of hierarchical or Gestalt-like processes from the dynamics of activity generated by these elements (i.e., a matrix). The development of these interconnected neuronal networks would follow along trajectories initially determined by genetic ("intrinsic") factors; however, subsequent factors that influence afferent ("extrinsic") activity could result in dynamic remodeling of this matrix. These changes in neuronal organization may be associated with experiences that might differ quite conspicuously after the remodeling, such as qualitatively different experiences of pain following neurological trauma [10,22]. Our employment of the "neuromatrix" hypothesis as a possible explanation for the different behavioral profiles seen in rats that received different pharmacological treatments following status epilepticus is in accordance with these hypotheses.

Any model that can reliably produce extensive multifocal damage to various spatially separated aggregates while selectively preserving other aggregates is optimal for investigating the relationship between brain and behavioral correlates. In theory, multifocal brain damage should have a greater capacity to produce more extensive and conspicuous impairments in general behavior. Although previous studies have shown histological evidence of extensive neuronal injury in the brains of rats treated with certain drugs following lithium–pilocarpine-induced seizures, behavioral sparing is often noted for various "cognitive functions" in rats that had been treated with ketamine following status epilepticus [8,10]. Conversely, rats that are treated with acepromazine following lithium–pilocarpine-induced seizures show extreme impairment in a broad range of learning and behavioral measures. Even though a direct histological assessment of the extent of neuronal damage in the brains of the rats from the various treatment conditions was not performed in this study, previous histological examinations of the brains of acepromazine- and ketamine-treated rats have revealed two distinct patterns of damage that are extremely consistent and remarkably similar across numerous experiments and between experimenters [2,6–9,23,24].

The results of this experiment support many of the basic features of the neuromatrix hypothesis. First, rats that were treated immediately following status epilepticus with ketamine showed preserved contextual fear learning that did not differ from that of nonseized control rats. However, acepromazine-treated rats showed a marked reduction or impairment in associating an aversive event (i.e., footshock) with a specific context, as inferred by the reduction in behavioral freezing during the 8-minute extinction test.

The observed reduction in contextual fear learning found in this study was not due to a nonspecific difference between groups with respect to their baseline locomotor activity that could interfere with place learning [25,26], nor was it a peculiarity in these animals' responsiveness to the footshock presentation during training, as inferred by midline chamber crossover,

postshock freezing behavior, and open-field testing measures. In other words, the deficit in fear learning was not due to a disparity in the overall motor behavior between the different groups but instead reflected a genuine deficit in the extent of fear learning that was consolidated after training in the contextual fear paradigm. It is also possible that the deficit observed on the testing day reflects a retrieval error of the unconditioned stimulus–conditioned stimulus associations that were learned during the training period.

Several of the neuroanatomical structures that are damaged following lithium–pilocarpine induced seizures are implicated in mediating either the acquisition or the expression of contextual fear learning [8,12]. Most notably is that damage to the basolateral amygdaloid nucleus can significantly impair the formation of context–footshock associations and this structure may play a role in the long-term storage of information relevant to fear conditioning [27,28,45,46]. Surprisingly, previous histological assessment of the brains of ketamine-treated rats indicated well over 90% damage to the basolateral amygdaloid complex, whereas acepromazine-treated rats show about 60% damage to this same structure [6,24,29]. This finding is striking and paradoxical when one considers the relatively preserved fear learning exhibited by ketamine-treated rats and the significant impairment found in acepromazine-treated rats during this study.

Contextual fear learning has previously been shown to be integrally dependent on the activation of NMDA receptors within the basolateral amygdala [30]. Involvement of the NMDA receptor is associated with epileptogenesis following status epilepticus and is crucial in the subsequent development of limbic epilepsy [31]. Therefore, these results imply that specific modulation of the NMDA receptor site during lithium–pilocarpine-induced seizures can significantly block the cascade of neuronally mediated events that are involved in both the induction of epileptogenesis and the behavioral consequences of epilepsy. In addition, although significant neuronal loss and gliosis are found within the basolateral complex this finding is consistent with the assumption that behavior is determined by neuronal activity and not by the absence of neurons. The remaining neurons (~10%) found in the basolateral amygdala of ketamine rats might be those neurons that are most important in the neural circuit mediating fear learning.

Although acepromazine-treated rats displayed less extensive damage to this structure in comparison to ketamine-treated rats, the specific loss of neurons that would have been preserved by the transient suppression of the NMDA receptor system during seizure induction probably never took place. Since these important neurons might be integral in the circuit mediating contextual fear learning and these very neurons may be lost in the brains of acepromazine-treated rats, our observation that contextual fear learning was impaired in these animals is expected. These findings are in accordance with the recent findings of McKay and Persinger [29] and with the unpublished observations of the first author.

Second, the results of the elevated-plus maze revealed that acepromazine-treated rats displayed a series of behaviors that would be highly suggestive of "lowered anxiety." An increase in the time spent in the open arms and greater intrusions into these areas would be labeled as "anxiolytic" by others [32,33]. The greater frequency of entry into the open arms and greater time spent in these arms by acepromazine-treated rats would support this claim. These results could suggest that the pattern of behavioral changes observed in these animals following seizure onset was associated with changes in the neural circuitry mediating emotional and anxiety-like behavior. Ketamine-treated animals did not display any peculiar responses on this measure and behaved in a manner identical to that of nonseized control rats.

Interestingly, we observed that when acepromazine-treated rats transversed the open arm areas of the plus maze extreme piloerection often occurred. Piloerection has been considered to be an accompanying peripheral autonomic response under stressful and highly emotional conditions [34]. Our behavioral observations were opposite to what one might expect. If acepromazine-treated rats indeed displayed a reduction in anxiety when entering the open arms, then the peripheral response that often accompanies anxiety should not have occurred. This suggests that there may be in fact two reasons why a rat might transverse the open arms of an elevated-plus maze [35]. One possibility is that the baseline level of anxiety in these rats has been lowered to such a point that it is no longer sufficient to inhibit the natural drive conflict of open-arm exploration. An alternative hypothesis is that the level of fear in these rats increased to such a point that motivation was enhanced in such a manner to cause these animals to seek escape from the apparatus by jumping off [35]. Interestingly, some studies have shown that amygdala-kindled rats tend to attempt escaping from the open arms of the elevated-plus maze by jumping during behavioral testing and have employed a similar interpretation of the behavioral data [36]. Furthermore, these researchers have hinted that this behavior might possibly be a form of excessive defensiveness [24].

Histological assessment of the brains of rats 30 days or more after they were treated with either acepromazine (ACE) or ketamine (KET) immediately following lithium–pilocarpine-induced seizures [6,8] has shown extensive and differential damage to various structures that are important in memory processes as well as in the mediation of fear and anxiety [35–39]. Moderate damage has been reported in the circuits mediating anxiety and affect. These include: anterior thalamic nucleus

(24% damage ACE, 5% damage KET); CA1 region (49% damage ACE, 20% damage KET); CA2 region (10% damage ACE, 3% damage KET); CA3 region (6% damage ACE, 6% damage KET); dentate gyrus (25% damage ACE, 6% damage KET); and perirhinal cortex (8% damage ACE, 19% damage KET). We suggest that differential activation of various receptor systems during status epilepticus could influence the extent of damage to structures important for both memory and anxiety processes.

That alteration in neuronal plasticity within the hippocampal circuitry can lead to significant impairment in both learning and memory as well as increased anxiety behavior in rats is well documented [40–42]. Since acepromazine-treated animals show considerably greater multifocal brain injury in the same hippocampal regions that are implemented in the modulation of the stress response (when compared with ketamine-treated and control animals), then functional disinhibition in the remaining neurons within these neural circuits (due to the large loss of inhibitory neuronal contact) might have led to a hyperreactive state within these animals [17]. Therefore, these rats, when placed in an unfamiliar context and exposed to a novel stimulus, may have responded to the stimulus in a highly emotional manner similar to that found in amygdala-kindled rats [35,36]. Our observation that acepromazine-treated rats would enter into, spend more time in, and often try to escape from the open arms of the plus maze apparatus seems to be in accordance with this explanation.

The report by Kalynchuk and colleagues [37] has shown that amygdala-kindled rats tend to display greater incidences of resistance to capture in a novel open field. This parallels our own findings for rats treated with acepromazine. These animals showed a high resistance to being captured compared with nonseized control rats. Specific damage to the medial amygdalar nucleus can result in an increase in the expression of defensiveness and anxiety in rats [43]. Since the medial amygdala receives dense cholingeric projections, activation of these afferents by pilocarpine during seizure activity may have affected this structure. Previous work [6,24] found that the medial amygdala nucleus displayed more extensive damage in the brains of acepromazine-treated (approximately 40% damage) compared with ketamine-treated rats (approximately 10% damage). Differences in the pattern and extent of damage to this structure could serve as an explanation for the behavioral differences observed between these two treatments.

The results of this experiment consistently demonstrated that specific pharmacological manipulation soon after seizure onset can significantly influence behavior and learning in rats tested following a recovery period. Specific timing and activation of different neuronal elements within the neurocircuitry mediating limbic seizures might also encourage the *emergence* of different behavioral patterns. This outcome would be highly dependent on how these pathways are both pharmacologically influenced and activated following the initial event. We further support previous findings found in our own laboratory [8–10] and those found by others [44] that administration of the NMDA antagonist ketamine immediately following status epilepticus may possess potential neuroprotective properties and can prevent many of the cognitive and behavioral impairments commonly observed in epileptic rodents. This finding indicates that ketamine may play a prospective role in the treatment and management of some forms of temporal lobe epilepsy. Further studies geared toward evaluating the clinical efficacy of this drug are well warranted.

Our findings suggest that conceiving the organization of the brain as a *functional matrix* is also operationally valid. Changes in groups of neurons whose functional connections play an integral role in the neuromatrix could produce qualitatively distinct changes in behavior. Furthermore, these results may also explain why individuals with clinically identical etiological manifestations of a seizure disorder or brain injury but treated using different pharmacological agents can display markedly different deficits in both behavioral and cognitive functioning.

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